Intro to Proteins III

I. 3-D Structures

A. Mechanisms of Folding

1. Primary structure contributes to protein three dimensional structure

2. Two compounds are useful in determining important interactions

a. β-Mercaptoethanol – reducing agent that reduces disulfide bonds

b. 8M Urea – disrupts noncovalent bonds

3. Step 1: Treat active enzyme with BME and 8M Urea 🡪 no activity

a. Protein has been denatured

4. Step 2: Remove both BME and 8M Urea 🡪 regains activity

a. Protein has “spontaneously” refolded

b. Removal of both chemicals allows folding to recur

5. Addition of BME 🡪 Urea 🡪 removal of BME

a. Results in random disulfide bond formation

b. Urea must be removed and a small amount of BME added to refold

6. Two important points:

a. O2 must be present to reform disulfides, must oxidize the free sulfhydryls

b. Release of free energy helps guide the enzyme into the correct shape

B. Nonrandom Folding

1. Levanthal’s Paradox – calculated time protein folding is far less than predicted

a. Levanthal assumes that the folding is random

2. Cooperative Transition – sharp transition from unfolded to folded

a. Denaturing experiments look at 50% unfolded protein

b. 50% fully folded, 50% fully unfolded 🡪 not 100% partially folded

3. Cumulative Selection – retention of partially correct intermediates

a. Correct, low energy folds are kept and remembered

C. Predicting 3-D Structures

1. *Ab initio* predictions

a. Prediction of structure without reference to known protein structures

b. Done using free energy calculations, but limited due to # of conformations

2. Knowledge-based methods

a. Prediction of structure based on sequence similar with known proteins

b. Done by working off of existing models and making some changes

3. Put simply, it is hard to predict

C. Post-translational Modifications

1. Phosphorylation – alcohols can be phosphorylated

2. Hydroxyl Group Addition to proline and lysine stabilizes collagen fibers

a. Vitamin C is important for this process, results in scurvy when deficient

3. Acetylation is important to help AA terminals resist degradation

4. Glycosylation – attaches sugars to Asn, Thr, or Ser

5. Collagen – makes up ~30% of total protein in the body

a. Made with hydroxyproline and hydroxylysine

b. Vitamin C is an essential cofactor for the prolylhydroxylase enzyme

c. Deficiency in vitamin C blocks collagen formation 🡪 scurvy

d. Mutations that cause every 5th Gly🡪Ala results in a distorted structure

i. Ala creates steric hindrance in the triple helix

D. Marginal Stability

1. Proteins are only marginally stable

2. This is biologically advantageous:

a. Optimal stability would prevent ligand binding & conformational change

b. Most proteins can do multiple tasks due to structural disorderliness

II. Protein Purification

A. Why is it important?

1. Determine AA sequence

2. Learn evolutionary relationships and importance of protein/sequences

3. Biochemical function

4. Obtain crystal and x-ray data that help picture tertiary structure

a. Relation between form and function

B. Differential Centrifugation

1. First step in purification

2. Homogenize cells and centrifuge at varying speeds/times

3. Allows for separation of parts of the cell and the soluble proteins

C. Dialysis

1. Separates larger molecules from smaller ones

2. Concentrated solution placed in porous bag which is then placed into a buffer

3. Salts/small molecules diffuse into buffer solution, leaving behind large molecules

D. Gel-Filtration Column Chromatography

1. Separates based on size

2. The column contains beads with a porous matrix that small molecules get stuck in

3. Larger molecules easily pass between beads and elute first

E. Affinity Chromatography

1. If possible, it is the best choice – high purity and yield

2. Separates and purifies proteins based on binding affinity for a specific ligand

3. Beads in the column have the ligand covalently attached

a. Proteins bind to the bead-ligands while other proteins elute out

b. Protein of interest is eluted with the addition of free ligand

F. Ion Exchange Columns

1. Separate proteins based on charge

2. Polymer beads have charged functional groups attached

a. Cation Exchange – Negative functional groups

b. Anion Exchange – Positive functional groups

3. Opposite charged proteins stick to the beads and move slower

4. Like charged proteins repel and move through the column quickly

G. High Pressure Liquid Chromatography (HPLC)

1. Can be used for almost all column techniques

2. Has a high resolving power and rapid separation

H. Determining Yield and Specific Activity

1. Yield – amount of pure protein collected

2. Specific activity – (activity of the protein/(amount of protein)

3. SDS-PAGE

a. SDS – Detergent that denatures a protein

b. PAGE – Polyacrylamide gel electrophoresis

c. SDS-PAGE separates in a gel based on size

i. Smaller proteins travel farther through the acrylamide matrix

4. After each method of purification, check specific activity (equation above)

5. Very high specific activity implies relatively pure protein

I. Isoelectric Focusing Gel

1. Separation based on pI of the protein

a. pI = pH at which protein is electrically neutral

b. Electric current run through a gel with a pH gradient

c. Protein stops moving once it reached its pI

J. Two-Dimensional Electrophoresis

1. Separates by pI and size

a. The extract is first separated on an isoelectric focusing gel

b. The gel is then run using SDS-PAGE to separate again based on size

K. Mass Spectrometry

1. Separates based on mass

2. Can tell content of a sample, but cannot be used as a purification technique

III. Determining Protein Sequence

A. Determining the AA composition

1. Hydrolyze protein in 6N HCl at 110°C for 24 hr

2. Separate by ion exchange chromatography

3. Quantify each protein by reaction with ninhydrin and the absorbance

a. Ninhydrin complexes with AAs to provide absorbances

B. Determine AA sequence by Edman Degradation

1. Reaction protein with phenyl isothiocyanate

a. Reagent reacts with N-terminal AA and removes it, forming a PTH-AA

b. Based on structure of resulting PTH-AA, AA at that position is determined

c. Reaction always occurs at N-terminal

C. Why?

1. Compare to known sequences to see if it corresponds to a protein family

2. Comparison between species yields info about evolutionary pathways

3. Find structural or functional motifs in the sequence

4. Sequences allow Ab for a protein to be developed for research purposes